Stichting voor Plantenbiotechnologie en -Weefselkweek (SVPW)
Society for Plant Biotechnology and Tissue Culture
www.svpw.nl info@svpw.nl KvK nr. 89069528 NL42INGB0004240007 BTW nr NL864869897B01

SVPW Fall symposium, Friday, December 8th, 2023

At: Ontmoetingscentrum **Zwijnaarde**, Dorpsstraat 31 – 9052 Gent (B)

10:00 **Registration, coffee / tea & meet-up**

10:55 **Opening by prof. dr. Remko Offringa and prof. dr. Danny Geelen**

11:00 **Dr. Steffen Vanneste** – **Ghent University**
A chemical approach towards understanding adventitious rooting

11:30 **Prof. dr. Hans de Jong** – **Wageningen University & Research**
A scientific journey of a variegated EMS tomato leads to an FtsH-like protein precursor and an epigenetic regulation

12:00 **Saba Taheri Msc.** – **Ghent University**
Controlled release of plant growth regulators in in vitro tissue culture using nanoparticles

12:30 **Dr. Renze Heidstra** – **Wageningen University & Research**
Regeneration through root stem cell factors

13:00 **Lunch & meet-up**

14:00 **SVPW info and questionaire**

14:30 **Dr. Kim Boutilier** – **Wageningen University & Research**
Brassinosteroid limits competence for somatic embryogenesis

15:00 **Vincent Jalink MSc**.– **Phenovation B.V.**
Unveiling the Unseen: Exploring the Hidden World of Plants with Imaging Technology in Research

15:30 **Coffee / tea break**

16.00 **Dr. Tom Eeckhaut** – **Flanders Research Institute for Agriculture, Fisheries and Foods (ILVO)**
Innovative breeding strategies: how in vitro regeneration expands a breeders' toolbox

16:30 **Dr. Devang Mehta** – **KU Leuven**
Closing gaps in plant chronobiology: from genome to proteome and from molecules to future geographies

17:00 **Closing drinks and meet-up**

Your contribution for attending the symposium is € 30, payable – preferably - in advance at IBAN: NL42INGB0004 2400 07, to the attention of Stichting SVPW
or via this link: https://www.ing.nl/particulier/betaalverzoek/index.html?trxid=twMQohsFzYOWldNZM1vdifjtIBgAdafH .or this QR-code:
or in cash at the symposium. This will mainly cover the costs of the lunch, coffee/tea andclosing drinks.
The printed day program and abstracts will be available at the symposium.

Please subscribe before December 4th via info@svpw.nl or www.svpw.nl

Secretariaat SVPW: Dr. Ir. H.C.H. Schoenmakers, Franseweg 26a, 3921DH Elst; tel. 0318-472912

Summaries of the lectures on the SVPW fall symposium, Friday, December 8th, 2023, Ghent (B)

A chemical approach towards understanding adventitious rooting

Dr. Steffen Vanneste – Ghent University

Lateral root (LR) branching and adventitious root (AR) formation are agronomic important traits determining crop yield and the efficiency of clonal propagation. While the initiation of LR has been studied intensively, the ontogeny of AR and its relationship to the initiation of LRs remain largely unknown. We identified from a collection of small molecule named Hypocotyl Specific Adventitious Root INducer (HYSPARIN; HYS) based on a strong AR inducing capacity without pronounced effects on primary root growth and lateral root branching. We found that HYSPARIN activates the pericycle in Arabidopsis hypocotyls, yet is not a typical auxin. Using this tool we started to uncover molecular components of the mechanism by which light inhibits adventitious rooting.

A scientific journey of a variegated EMS tomato leads to an FtsH-like protein precursor and an epigenetic regulation

Prof. dr. Hans de Jong – Wageningen University & Research, in cooperation with Kasetsart University, Kamphaeng Saen, Thailand

Leaf variegation is an intriguing phenomenon observed in many plant species. However, questions remain on its mechanisms causing patterns of different colours. In this study, I describe a tomato plant detected in an M2 population of EMS mutagenized seeds, showing variegated leaves with dark, medium, and light green, and white sectors. Cells and tissues of these classes, along with wild-type tomato plants, were studied by light, fluorescence, and transmission electron microscopy. We also measured chlorophyll a/b and carotene and quantified the variegation patterns with a machine-learning image analysis tool. We compared the genomes of pooled plants with wild-type-like and mutant phenotypes in a segregating F2 population to reveal candidate genes responsible for the variegation. This bulk segregant analysis identified SNP and InDels via bioinformatic analysis. The mutation mapping bioinformatic pipeline revealed a region with three candidate genes in chromosome 4, of which the FtsH-like protein precursor carries an SNP that we consider the causal variegated phenotype mutation. Phylogenetic analysis shows the candidate is evolutionary closest to the Arabidopsis Var1. The synonymous mutation is at a miRNA binding site, potentially disrupting the photoprotection mechanism and thylakoid development, resulting in leaf variegation.

Controlled release of plant growth regulators in in vitro tissue culture using nanoparticles

Saba Taheri Msc. – Ghent University

Plant growth regulators (PGRs) play a critical role in plant tissue culture. Traditionally, PGRs are dissolved in the culture medium, resulting in a uniform distribution throughout the plant tissues in contact with the medium. However, at different stages of plant development, the presence of spatial and temporal gradients of hormones and signalling molecules is essential. Unfortunately, achieving such gradients by simply dissolving these substances in the medium is often difficult. The implementation of a controlled and gradual release system for PGRs is a promising approach to control *in vitro* plant development. Among the most promising candidate carriers for this purpose are mesoporous micro $CaCO_3$ particles. These particles can release PGRs in a gradual manner, creating the desired spatial and temporal gradients within plant tissues. This innovative research combines plant *in vitro* technology with nanobiotechnology to create $CaCO_3$ particles with different physical properties that can influence the uptake and release profiles of PGRs. In this

study, the effect of encapsulation is thoroughly investigated. It will then be subjected to bioassays using model species and applied to the *in vitro* regeneration of meristems from a wide range of plant species. This technology has the potential to become a unique and powerful tool in plant biotechnology, enabling researchers to manipulate and enhance plant growth with unprecedented precision.

Regeneration through root stem cell factors

Dr. Renze Heidstra – Wageningen University & Research

Plants have the ability to regenerate new tissue and organs upon damage. The regeneration process involves a cascade of inductive cues leading to reprogramming, proliferation, competence acquisition, and development of new organs from somatic cells. Experiments in Arabidopsis demonstrate that during regeneration, cells are first persuaded to change fate towards root stem cell-like identity and subsequently are reprogrammed to acquire shoot fate. All these events are commonly triggered by the application of auxin and cytokinin in tissue culture methods. However, hormone application inevitably induces excessive gene induction thereby overclouding the identification of those genes truly required for the successive steps in regeneration. We aim to bypass the hormone treatment and directly induce root stem cell factors as a tool to induce regeneration in a way similar to the induced pluripotent stem cell concept in the animal field. To allow the induction of genes as well as putative uncoupling of the regeneration stages, we investigate the application of multiple inducible transactivation systems simultaneously. The induction of stem cell factors then allows us to tackle regeneration recalcitrance in plants by studying the variation in natural Arabidopsis accessions, by determining the intermediate downstream target genes and by its translation to other plant species.

Brassinosteroid limits competence for somatic embryogenesis

Dr. Kim Boutilier – Wageningen University & Research

Somatic embryogenesis (SE) is a type of totipotent growth in which embryos develop from vegetative cells. SE can be induced in vitro by exposing explants to exogenous growth regulators, in particular the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D). In arabidopsis, 2,4-D treatment induces SE from a wide range of explants, but these explants show large differences in their competence for SE. We performed a chemical screen to identify novel compounds targeting pathways that enhance the competence of mature arabidopsis embryos for 2,4-D-induced SE. Screening of the LATCA small compound library identified 4-chloro-N-methyl-N-(2-methylphenyl) benzenesulfonamide (brassinomide, BRM) as a powerful enhancer of SE. Pharmacological, genetic and biochemical analysis indicate that BRM is a novel brassinosteroid biosynthesis inhibitor, and that BRM treatment promotes auxin- and ABA hypersensitivity during SE. These results uncover a new role for brassinosteroids as negative regulators of cell totipotency.

Unveiling the Unseen: Exploring the Hidden World of Plants with Imaging Technology in Research

Vincent Jalink MSc.– Phenovation B.V.

In the field of plant research, we've long depended on what we can directly see with the unaided eye. Yet, the landscape of plant research is undergoing a profound transformation thanks to the advent of advanced imaging technology. While imaging technology has been integrated into various aspects of plant research over the past decade, it has yet to fully penetrate the domain of tissue culture research. In this presentation, we'll demonstrate the potential seamless integration of these imaging techniques into tissue culture research, highlighting the immense benefits they offer to a wide array of research topics.

Innovative breeding strategies: how in vitro regeneration expands a breeders' toolbox

Dr. Tom Eeckhaut – Flanders Research Institute for Agriculture, Fisheries and Foods (ILVO)

Any in vitro based breeding technique depends on the availability of a sufficiently performant regeneration system. This will be demonstrated with some case studies. Coculture of plant material with wild strains of *Rhizobium rhizogenes* can produce so-called hairy roots, that contain *rol* genes. In vitro induction and regeneration of these roots is indispensable for the production of plants with *rol* genes that often exhibit interesting phenotypes. Protoplast-based CRISPR gene targeting holds some advantages over other approaches, but depends on a cell-to-plant regeneration protocol that is often very genotype specific. Asymmetric protoplast fusion may be used as a tool to transfer mitochondria between species, leading to CMS in the acceptor species. Regeneration of the desired cybrids is not evident because of cytoplasmome instability and preferential regeneration of unfused protoplasts. Over the last years, we have developed protocols for *Chrysanthemum*, *Solanum*, *Cichorium* and other species to enable the incorporation of the aforementioned tools in their breeding schemes as complementary tools with traditional breeding.

Closing gaps in plant chronobiology: from genome to proteome and from molecules to future geographies.

Dr. Devang Mehta – KU Leuven

Climate-change is causing a gradual northward shift in the growing regions of many crop plants. Previous research by us and others has found that plants use their circadian clock to sense changes in their geographical environment, such as differences in light intensity and quality to shape their growth and development. As climate change forces agriculture to expand into more Northern latitudes, we need to understand how the clock functions in different environments and how we can tweak it to engineer future-proof crops. My newly established research group is attempting to decipher how plant circadian clock processes external light signals that change with latitude, building upon our recent discovery that the circadian clock is key to how plants respond to changes in twilight length. We are also researching how the circadian clock subsequently controls gene regulation to impact a variety of biological processes. We know from decades of fundamental research that the circadian clock in plants consists of highly interconnected transcriptional-translational feedback loops that control the expression of approximately 40% of all genes. However, little is yet known about how these rhythms in transcription translate to rhythmic protein expression. By developing a new mass-spectrometry technique for quantitative proteomics, we are producing a circadian protein atlas in plants, providing unprecedented global insight into the timing of gene expression by the clock. We are now seeking to use such new proteomics approaches to characterize how plant chronobiology responds to environmental change with the aim of engineering latitudinal adaptability in crop plants.

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